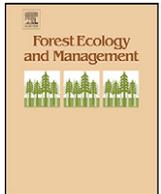




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## Coast live oak, *Quercus agrifolia*, susceptibility and response to goldspotted oak borer, *Agrilus auroguttatus*, injury in southern California

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### ABSTRACT

Oak mortality is often associated with a complex of decline factors. We describe the morphological and physiological responses of coast live oak, *Quercus agrifolia* Née, in California to an invasive insect, the goldspotted oak borer (GSOB), *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), and evaluate drought as a potential inciting factor. Morphological traits of 356 trees were assessed and physiological traits of 70 of these were monitored intensively over one growing season. Morphological characteristics of tree health included crown thinning and dieback; bole staining resulting from larval feeding; density of GSOB adult exit holes; and holes caused by woodpecker feeding. These characteristics were used to rank GSOB infestation/injury into four classes, and taken together, they explained 87% of the variation in a principal component analysis. Drought stress on various size/age and infestation classes of *Q. agrifolia* was measured by assessing branchlet pre-dawn and solar noon xylem water potential, leaf cell turgor potential, and water use efficiency over one growing season. Both morphological and physiological traits were highly variable in mature and old growth trees. Early summer plant water status (branchlet xylem water potential and water use efficiency) was similar between uninfested and newly colonized trees, suggesting that GSOB are not pre-selecting drought-stressed *Q. agrifolia* for oviposition. By late summer, leaf water and cell turgor potentials were lower in infested than in uninfested mature trees, suggesting that GSOB infestation causes drought stress in these trees. Among the tree size/age classes, infested old growth trees exhibited the greatest change in water use efficiency over the growing season, and showed greater morphological injury symptoms of decline than infested mature trees. Morphological attributes of decline in *Q. agrifolia* associated with GSOB were correlated weakly with increasing physiological drought stress among infestation classes of trees. We propose that the collection of morphological responses of *Q. agrifolia* to GSOB described here can be used to monitor the future expansion of the GSOB distribution as well as the GSOB-induced decline of *Q. agrifolia* in California.

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### 1. Introduction

Worldwide, the decline of oaks is a subject of great interest and concern (Führer, 1998; Thomas et al., 2002; Kamata and Futai, 2010). Elevated levels of oak mortality are commonly associated with a complex of predisposing, inciting, and contributing factors (Manion, 1981; Pederson, 1998). Predisposing factors, or long-term

stresses, such as dense forest stands, air pollution, and topography, enhance the susceptibility to inciting factors, which are typically short in duration and associated with drought, frost injury, or insect defoliation (Staley, 1965; Nichols, 1968; Stringer et al., 1989). Contributing factors such as infections by root disease and canker fungi, or colonization by phloem/wood boring insects can ultimately lead to oak mortality (Clinton et al., 1993; Starkey et al., 2004). *Agrilus* spp. (Coleoptera: Buprestidae) phloem/wood borers have frequently played a role in oak mortality worldwide (Dunbar and Stephens, 1975; Wargo, 1977; Haack and Acciavatti, 1992; Moraal and Hilszczański, 2000; Vansteenkiste et al., 2005; Sierpinski and Hilszczański, 2006; Hilszczański and Sierpinski, 2007), but have often been considered to be secondary or contributing factors.

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The goldspotted oak borer (GSOB), *Agrilus auroguttatus* Schaeffer<sup>2</sup>, is a flatheaded borer that is believed to be introduced to San Diego County, California via transport of infested firewood from a known population in southeastern Arizona or a suspected population in northern Mexico (Coleman and Seybold, 2008a, 2010, 2011; Hespeneide and Bellamy, 2009). The larvae of this beetle mine in the phloem, cambium, and primarily in the outer xylem of the main stem and larger branches of several species of oaks (Coleman and Seybold, 2008b). This phloem/wood borer is killing oaks (Coleman and Seybold, 2008a), and its population is expanding radially in San Diego Co. (Coleman and Seybold, 2011). In California, it affects three indigenous oak species: coast live oak, *Quercus agrifolia* Née; California black oak, *Q. kelloggii* Newb.; and canyon live oak, *Q. chrysolepis* Liebm., which dominate 1.5 million ha of California forest landscape (Gaman and Firman, 2006). In southeastern Arizona, the native hosts of GSOB appear to be Emory oak, *Quercus emoryi* Torrey, and silverleaf oak, *Q. hypoleucoides* A. Camus, but the level of mortality that indigenous populations of GSOB cause in these hosts is much lower (Coleman and Seybold, 2011). The success of GSOB as a mortality agent on the three species of oaks in California has been attributed to the novelty of the host relationships (i.e., an absence of co-evolved host resistance) and to the absence of a significant natural enemy complex (Coleman and Seybold, 2011). Only the indigenous acorn woodpecker, *Melanerpes formicivorus* (Swainson) and Nuttall's woodpecker, *Picoides nuttallii* (Gambel) (both Piciformes: Picidae) have been observed feeding frequently on GSOB in California.

Within infested stands in California, oaks greater than 12 cm in diameter at breast height (DBH, at 1.47 m) are susceptible to GSOB injury and mortality. In some stands, all mature and old growth oaks are succumbing to GSOB infestation. Without the ability to enforce firewood transport restrictions and to limit intrinsic and human-assisted beetle dispersal, we expect continued expansion of the range and invasion of California, including perhaps the Sierra Nevada, Coast, and Transverse mountain ranges (Coleman and Seybold, 2009).

Oak decline and mortality in San Diego Co. were first observed in late 2002, after three consecutive years of moderate (1999–2001) and a single year of acute (2002) regional drought (Grulke et al., 2009). The primary species sustaining this mortality have been *Q. agrifolia* and *Q. kelloggii*. After years of misdiagnosis, the cause of the mortality was attributed to GSOB in 2008 (Coleman and Seybold, 2008a). As of 2010, the beetle has killed approximately 21,500 oaks over an area of 4903 km<sup>2</sup>. We hypothesized that successful initial colonization and tree-to-tree spread of GSOB were related to tree drought stress (Coleman and Seybold, 2008a), as has been suggested for other species of Buprestidae known to attack the main stem of hardwoods (Dunn et al., 1986; Katovich et al., 2000; Moraal and Hilszczański, 2000).

Coincident with an understanding the role of drought stress in successful colonization of oak by GSOB, a definitive morphologically-based, tree health rating system is needed to evaluate infested oaks. Symptom-based rating systems for monitoring the progression of mortality agents in California forests have been published for pitch canker disease (Storer et al., 2002) and sudden oak death (McPherson et al., 2005, 2010a,b). We described four morphological attributes that characterize the response of *Q. agrifolia* to GSOB infestation (Coleman and Seybold, 2008b): crown

thinness; bole stain resulting from extensive larval feeding; D-shaped exit holes associated with adult emergence; and distinctive woodpecker foraging holes in the outer bark. When sufficiently quantified and validated, these attributes might provide guidelines for symptom recognition and a tree health rating system for use by land managers to quickly and consistently identify damage attributable to GSOB. The guidelines would be useful in planning subsequent management actions (e.g., hazard tree removal or insecticide treatment of high-value trees) or as a ground survey tool to assist in characterizing the future expected expansion of the zone of infestation in California.

This interdisciplinary study was initiated to (1) completely describe and quantify morphological traits that collectively and definitively attribute decline of *Q. agrifolia* to GSOB injury; (2) test whether newly infested oaks in a stand had less favorable plant water status than uninfested trees (i.e., does tree drought stress play a role in the likelihood of a new attack by GSOB?); (3) determine whether trees with increasing severity of GSOB symptoms also had greater drought stress (i.e., is drought stress a response to and the cause of tree mortality in GSOB infestation?); and (4) test whether morphological and physiological symptoms of decline were correlated.

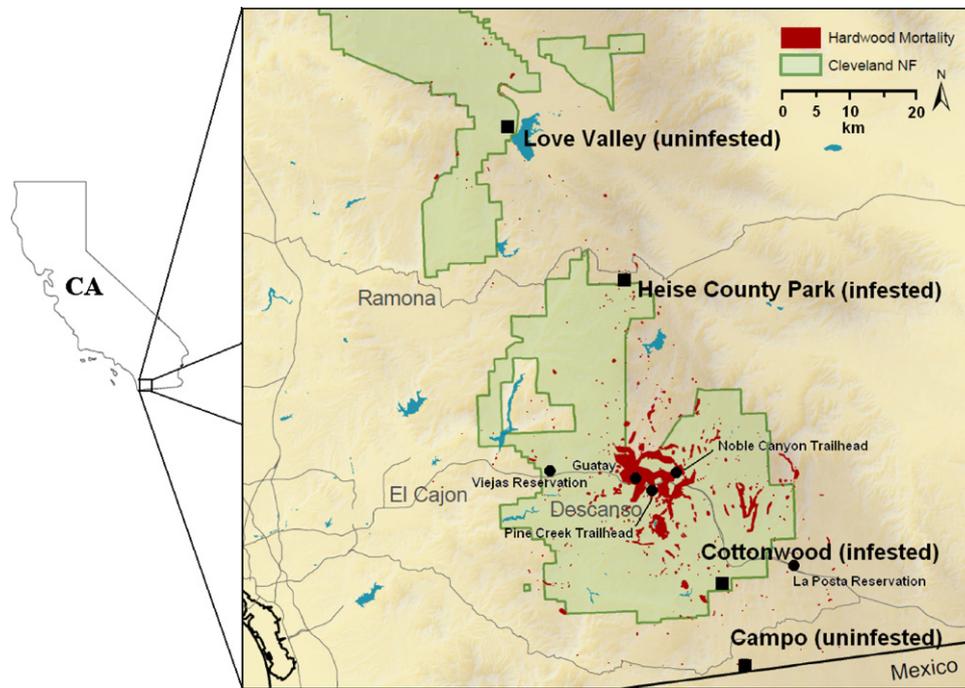
## 2. Materials and methods

### 2.1. Site selection

During April and December 2009, 356 *Q. agrifolia* trees were assessed on five study sites (Viejas and La Posta Reservations, Pine Creek and Noble Canyon Trailheads (both Cleveland National Forest), and Guatay, California, Fig. 1) for morphological responses to GSOB within the infested area. At these sites, the current infestation rates for *Q. agrifolia* and level of injury from GSOB were determined by a ground survey. The survey included old growth (>75 cm DBH) ( $N = 234$ ), mature (25–75 cm DBH) ( $N = 104$ ), and pole-sized (<25 cm DBH) ( $N = 18$ ) *Q. agrifolia*.

In order to evaluate the morphological responses of *Q. agrifolia* to GSOB in the context of its physiological status, one forest stand was selected north (Love Valley Meadow, Palomar Ranger District, Cleveland National Forest (33°15'16.24" N, 116°46'26.48" W, elevation 1070 m)) and one was selected south (rural/residential area, Campo, California (32°36'32.61" N, 116°28'07.66" W, elevation 808 m)) of the current margin of the infested area (Coleman and Seybold, 2011) (8.5 km north and 4 km south, respectively). We anticipated collecting background data from these sites prior to their invasion by GSOB. In contrast, two other stands were selected for physiological study within the zone of GSOB infestation (William Heise County Park, Julian, California (33°02'36.83" N, 116°35'09.58" W, elevation 1270 m) and Cottonwood Creek Riparian Area, Descanso Ranger District, Cleveland National Forest (33°42'01.88" N, 116°29'21.07" W, elevation 951 m)) (Fig. 1). Outside the infested zone (i.e., at Love Valley Meadow and Campo), five old growth (>80 cm DBH), five mature (50–80 cm DBH), and five pole-sized (<50 cm DBH) *Q. agrifolia* were selected for this intensive study (Table 1). Within the infested zone, five mature (25–80 cm DBH) *Q. agrifolia* were selected from each of four GSOB infestation classes (uninfested, and lightly-, moderately-, and severely-infested) for intensive physiological study. The criterion used to establish the level of infestation was the degree of crown thinning (uninfested: healthy; lightly infested: twig dieback and/or minor thinning and dieback; moderately infested: moderate branch thinning and dieback; and severely infested: severe branch thinning and dieback) (Coleman and Seybold, 2008b). Sites were visited four times from late May through mid-November 2009 to assess water status of the trees (see below). Initial assessments for

<sup>2</sup> Populations of this borer from southeastern Arizona were originally named *A. auroguttatus* Schaeffer (Schaeffer, 1905; Fisher, 1928); synonymized with *A. coxalis* Waterhouse (Hespeneide, 1979); given subspecific status as *A. coxalis auroguttatus* Schaeffer (Hespeneide and Bellamy, 2009); and then recently returned to their original taxonomic standing as *A. auroguttatus* Schaeffer (Hespeneide et al., 2011). The introduced population in California is *A. auroguttatus*.



**Fig. 1.** Location of nine study sites relative to oak mortality associated with goldspotted oak borer (GSOB), *Agrilus auroguttatus*, during 2002–2009 on and adjacent to the Descanso and Palomar Ranger Districts of the Cleveland National Forest, San Diego Co., California, USA.

GSOB infestation and injury were conducted in March 2009 when sites were established prior to GSOB adult emergence. The average tree density and basal area across the four sites were 22 ha<sup>-1</sup> and 42 m<sup>2</sup> ha<sup>-1</sup>, respectively.

Although the four intensively studied stands had similar relationships between diameter and height growth (C. Godinez, unpublished data), the stands differed in structure and site water regime. As these sites are all approximately 60 km east of the Pacific Ocean, they receive frequent morning marine fogs from May

through mid-August. Trees at Love Valley Meadow (hereafter Love Valley) were openly distributed in a narrow band of mixed *Q. agrifolia* and Engelmann oak, *Q. engelmannii* Greene, around an open grassland with low levels of spring grazing. Love Valley is located on a perched tableland on the southeastern slope of Palomar Mountain and is well drained. Trees at William Heise County Park (hereafter Heise) were represented by a high density, mixed stand of *Q. agrifolia*, *Q. kelloggii*, and *Q. chrysolepis* interspersed with Coulter pine, *Pinus coulteri* D. Don (Fig. 2). Because the intensively studied trees

**Table 1**

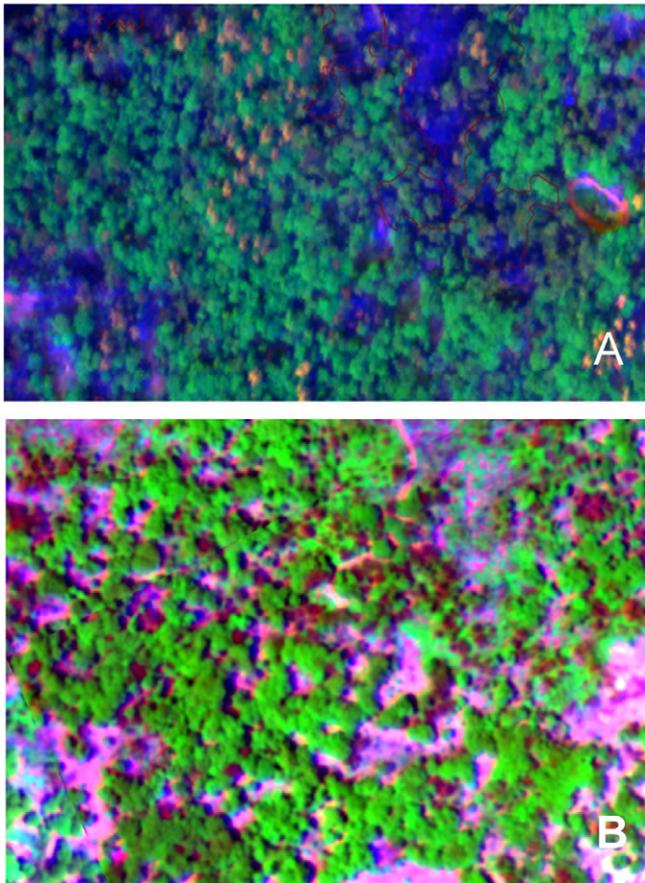
Summary of morphological attributes of coast live oak, *Quercus agrifolia*, with and without injury from the goldspotted oak borer, *Agrilus auroguttatus*, across the zone of infestation and for uninfested and infested intensively studied sites in San Diego County, California<sup>a</sup>.

	N	Crown thinning (rating, 1–5)	Bark staining (rating, 0–4)	Exit holes (rating, 0–3)	Woodpecker (rating, 0 or 1)	Diameter (cm) <sup>b</sup>
<b>Zone of infestation surveys</b>						
Mature (25–75 cm dia.)	104	1.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	50.8 ± 1.2
Old (>75 cm dia.)	234	1.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	110.5 ± 2.7
Uninfested	146	1.2 ± 0.0	0.1 ± 0.0	0	0	64.2 ± 3.3
Infested	210	1.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.3 ± 0.0	82.4 ± 3.2
<b>Love Valley Meadow (uninfested)</b>						
Pole (<50 cm DBH)	5	1.0 ± 0.0	0	0	0	28.6 ± 3.1
Mature (50–80 cm DBH)	5	1.3 ± 0.3	0	0	0	58.9 ± 2.8
Old growth (>80 cm DBH)	5	1.0 ± 0.0	0	0	0	89.8 ± 6.2
<b>Campo (uninfested)</b>						
Pole	5	1.4 ± 0.4	0	0	0	39.3 ± 2.9
Mature	5	1.6 ± 0.4	0	0	0	58.5 ± 5.4
Old growth	5	1.4 ± 0.2	0	0	0	146.8 ± 10.5
<b>Heise County Park (infested)<sup>c</sup></b>						
Uninfested	5	1.2 ± 0.2	0	0	0	54.6 ± 6.8
Light	5	1.8 ± 0.2	0	0.5 ± 0.3	0	49.3 ± 2.1
Moderate	5	2.2 ± 0.5	2.0 ± 0.7	0.8 ± 0.4	0.6 ± 0.2	52.9 ± 3.6
Severe	5	3.2 ± 0.4	3.0 ± 0.0	1.6 ± 0.4	1.0 ± 0.0	61.2 ± 0.7
<b>Cottonwood (infested)<sup>c</sup></b>						
Uninfested	5	1.2 ± 0.1	0	0	0	55.2 ± 4.5
Light	5	2.3 ± 0.3	0.2 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	58.5 ± 6.2
Moderate	5	2.2 ± 0.6	0.2 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	48.6 ± 1.5
Severe	5	3.6 ± 0.5	1.8 ± 0.7	0.8 ± 0.5	0.4 ± 0.2	52.6 ± 5.0

<sup>a</sup> Table entries given are mean ± S.E.

<sup>b</sup> At breast height (DBH, measured at 1.47 m).

<sup>c</sup> At the two infested sites used for the intensive study, only mature (25–80 cm DBH) *Q. agrifolia* were selected among each infestation/injury class.



**Fig. 2.** Aerial imagery of William Heise County Park, San Diego Co., California, showing change in oak mortality of the same area in 2003 (A) and 2008 (B). The influence of the Cedar fire in 2003 is inscribed by polygons in the upper image (A). Light beige canopies in the 2003 image were pine that died in response to a drought (1999–2002). Dark rusty brown canopies in the 2008 imagery were coast live oak that succumbed to goldspotted oak borer, *Agrilus auroguttatus*.

were several hundred meters from the camping area, the impact of the park campground on these trees was low. The Cedar Fire burned into the area in 2003, and two of the intensively studied trees had minor scorching (<10%) at the base of their boles. The Cottonwood Creek site (hereafter Cottonwood) was topographically low, and *Q. agrifolia* dominated a moderately open stand within 200 m laterally and 15 m vertically above the creek. *Quercus agrifolia* also dominated the Campo site, and the trees were openly distributed on a well drained, west-facing slope. Some of the trees were adjacent to the site of a demolished building, and these trees had likely experienced increased, localized soil compaction. During establishment of the study, we observed a low density of recent GSOB attacks on two of the trees at Campo, reflecting the nascent southward expansion of infestation. These newly colonized trees were likely affected by soil compaction.

## 2.2. Morphological responses to GSOB injury

Morphological responses of *Q. agrifolia* to GSOB injury were used to develop a health rating system by adapting similar health rating techniques from congeners, the emerald ash borer, *A. planipennis* Fairmaire, and the bronze birch borer, *A. anxius* Gory (Katovich et al., 2000; Poland and McCullough, 2006) (Obj. 1). These attributes (Fig. 3) were assessed and recorded for each tree selected for intensive study, as well as for those in the zone of infestation survey. Bole DBH of each tree was also measured. Each tree crown was rated for thinness relative to healthy trees typical of the region. Crown thin-

ness was based on guidelines developed by the USDA Forest Service, Forest Inventory Analysis (FIA Program) (Schomaker et al., 2007); reported for *A. anxius*-injured trees (Katovich et al., 2000); and reported for the common stand exam (USDA Forest Service, 2003). We established five crown rating classes: (1) full, healthy crown with no apparent leaf loss; (2) minor twig dieback or light thinning, 10–25% leaf loss; (3) moderate twig dieback and thinning, 25–50% leaf loss; (4) severe dieback progressing to larger branches, >50% leaf loss; and (5) complete loss of leaves, tree mortality. The first four crown classes are hereafter referred to as healthy, lightly, moderately, and severely injured, respectively. Five stain rating classes were established for the lower portion (<2.5 m) of the primary bole: (0) no staining; (1) 1–5 stained areas; (2) 6–10 stained areas; (3) >10 stained areas; and (4) staining present and bark cracking away from the xylem. Outer bark staining is indicative of advanced degradation of subcortical tissue associated with feeding by larval GSOB. This symptom has also been observed with other *Agrilus* spp. that injure hardwoods (Solomon, 1995; Vansteenkiste et al., 2005). We also established four D-shaped adult exit hole rating classes for the lower portion (<2.5 m) of the primary bole: (0) no exit holes; (1) 1–9; (2) 10–25; and (3) >25 exit holes. Finally, woodpecker foraging holes were recorded as present or absent by examining the bole for chipped/exposed brick-red phloem that revealed dark-colored GSOB larval galleries or pupal cells just below the outer bark surface where the birds located their prey (Coleman and Seybold, 2008a,b).

## 2.3. Soil moisture

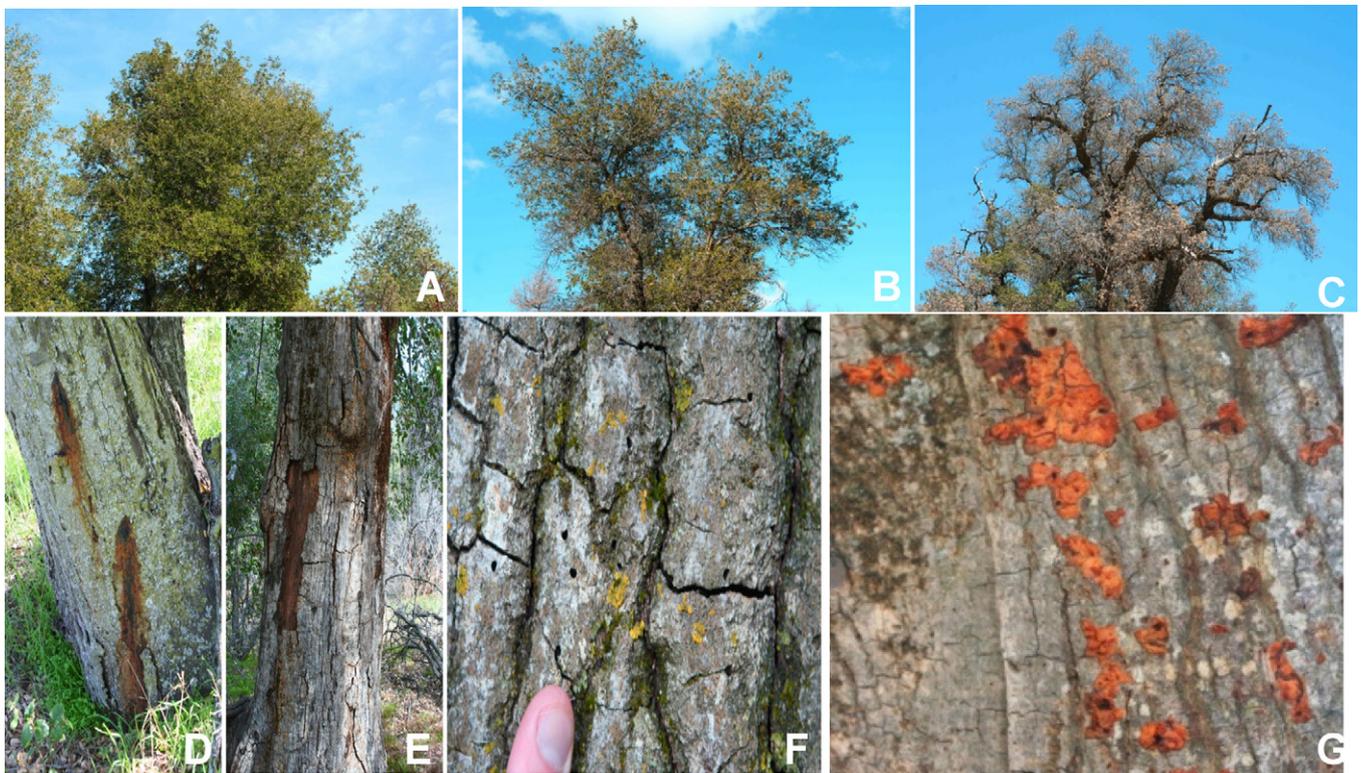
Soil moisture was measured over the growing season concurrently with measurements of tree drought stress (see below). A small pit (20 cm × 20 cm × 50 cm) was excavated, and 2 subsamples of approximately 60 g each were collected from the bottom of the pit, and transferred into soil tins. The amount of soil filled each tin and the tins were sealed for storage. Next, soil was sieved to pass 2 mm, and the sieved fraction was weighed to the nearest 0.01 g (wet weight), then oven dried for 4 d at 105 °C, and re-weighed (dry weight). Moisture content was expressed as percentage moisture of the dry weight:

$$\frac{(\text{wet weight}) - (\text{dry weight})}{(\text{dry weight})} \times 100\% \quad (1)$$

## 2.4. Physiological responses to drought stress and GSOB injury

When evaluating drought stress in trees, low leaf water potential values alone are insufficient to infer stress. Moderate drought stress is defined as a decline in total leaf water potential that is accompanied by an increase in cell osmoticum, reduced stomatal conductance, and slightly reduced cell turgor (Levitt, 1980). Under conditions of moderate drought stress, the effect of reduced turgor on cell elongation may be detectable. Severe drought stress is defined by significant declines in total leaf water potential; significant increases in cell osmoticum; stomatal closure over a significant proportion of the day or growing season; and significant reduction in cell elongation growth. In its extreme, osmoticum increases sufficiently to disrupt cellular enzymatic function and results in cell death.

Water use efficiency (WUE,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) is another physiological metric of drought stress that incorporates both leaf photosynthetic carbon gain and transpirational water loss. When soil water is limiting, the plant optimizes gas exchange such that the most carbon is gained for the least water lost (high WUE) (Tardieu and Simmoneau, 1998). If drought stress is experienced during the previous day and a tree is carbon-limited, the stomata may open the following morning to regain carbon for only a couple of hours, but then may close for



**Fig. 3.** Illustration of tree health ranking assigned to coast live oak, *Quercus agrifolia*, crown thinning and dieback, bole staining, exit hole density, and woodpecker foraging holes specific to the goldspotted oak borer, *Agrilus auroguttatus*. The full range in crown thinning and dieback (rating 1–5; A: rating of 1, B: rating of 3, and C: rating of 5), bole staining from absent to bark stained and cracking (0–4; D: rating of 1 and E: rating of 4), D-shaped exit holes (0–3; F: rating of 3), and woodpecker foraging holes (present (G) or absent).

the rest of the day (low or no measurable stomatal conductance) (Goulden, 1996). Under these conditions, WUE is likely to be high initially with the first direct sun, then drop precipitously as the leaf is unable to maintain transpiration. During both the early and late summer, the differences between afternoon and morning WUE were evaluated for the various size/age classes and infestation classes of *Q. agrifolia* to illustrate differences in strategies for maintaining favorable plant water status.

At four times during the growing season, we measured changes in (1) pre-dawn and (2) solar noon branchlet xylem water potential ( $\Psi_{xPD}$  and  $\Psi_{xSN}$ , respectively); (3) leaf cellular osmotic potential,  $\Psi_{\pi}$  (and thus turgor potential,  $\Psi_p = \Psi_{xSN} - \Psi_{\pi}$ ); and (4) leaf WUE. These attributes and their variability were used to evaluate potential differences in vulnerability to drought stress among the three tree size/age classes; to examine whether oak water status differs between uninfested and newly infested trees (Obj. 2); and to describe water status of previously injured *Q. agrifolia* from different GSOB infestation classes (Obj. 3).

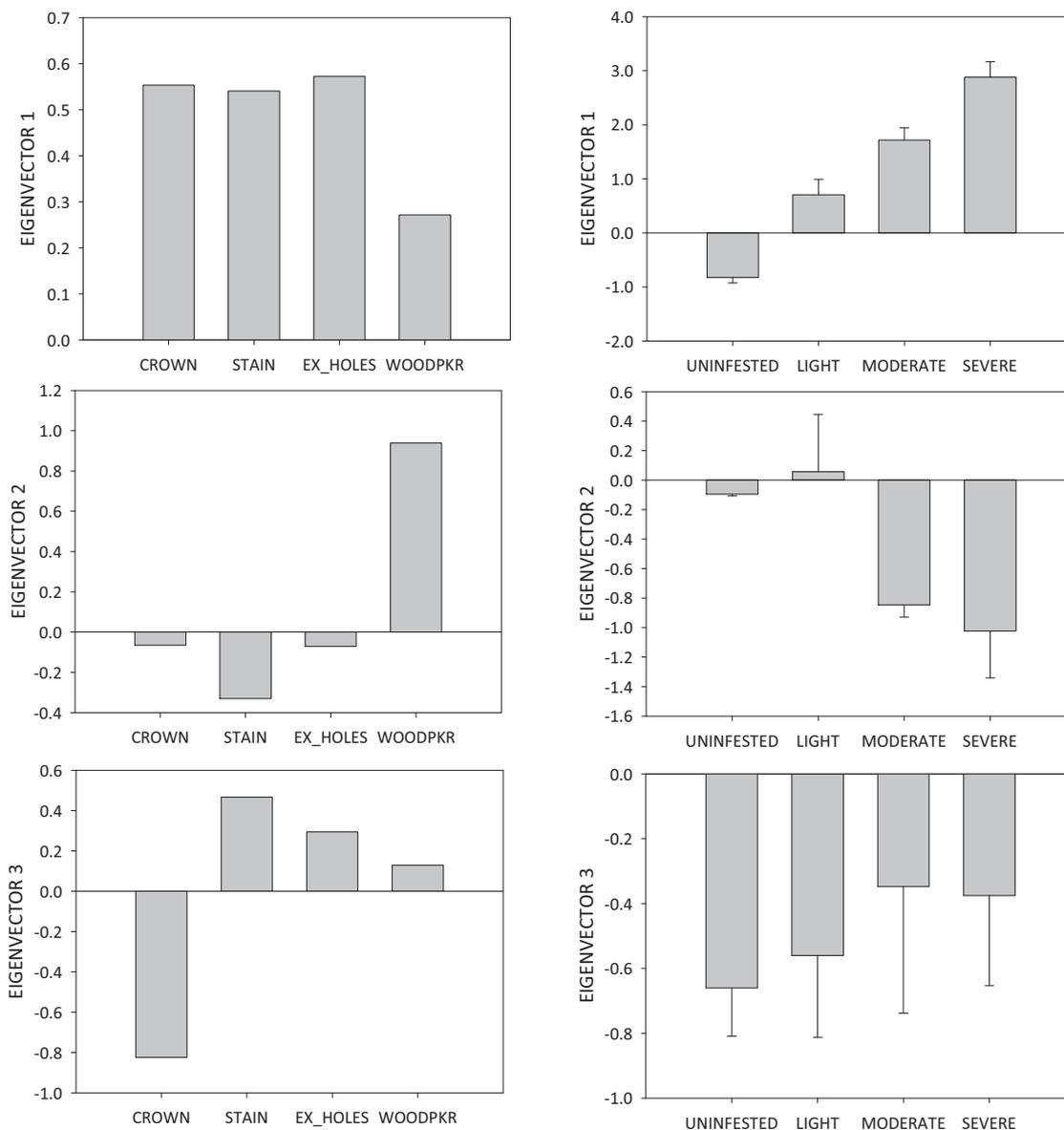
To measure  $\Psi_{xPD}$ , branches were subsampled in the morning from ~2 m above the ground before the tree canopy received direct sunlight, packaged in air-tight plastic bags with little air, and placed in a cooler until measured soon afterwards. Just before measurement, branchlet stems were trimmed with a razor blade, inserted into a pressure chamber (PMS, Inc., Corvallis, OR, USA). Pressurized  $N_2$  was applied until moisture was just apparent on the cut end (Pallardy et al., 1991). Branchlets retained 1 leaf age class during late May and early June measurements, and 2 leaf age classes subsequently. To measure  $\Psi_{xSN}$ , branches were subsampled at solar noon from mid-canopy (~4 m), taking care to ensure that the branch had experienced full sun for at least 0.5 h prior to sampling. The procedure for measurement was similar to that for pre-dawn measurements.

To determine osmotic potential  $\Psi_{\pi}$ , a subsample of leaves was used from the  $\Psi_{xSN}$  collection. Six 6 mm diameter hole punches were removed from ‘average’ leaves, wrapped tightly in aluminum foil, slipped into cryovials, and frozen in either liquid nitrogen or on dry ice. Samples were stored in a  $-80^{\circ}C$  freezer until osmotic potential was measured with a microvoltmeter (Wescor Dewpoint Microvoltmeter, Logan, UT, USA) and chamber psychrometers (model 75-1.5C, JRD Merrill Specialty Equipment, Logan, UT). A standard psychrometric approach was used to determine osmotic potential, by using a 25 s cooling period after 1.25 h equilibration in a  $24^{\circ}C$  water bath. Psychrometers were calibrated with three KCl solutions of differing molarity (Pallardy et al., 1991), across the expected range of osmotic potential.

Water use efficiency was used to demonstrate changes in plant strategy for maintaining favorable plant water status over the growing season for different tree size/age classes and among infestation levels. An open gas exchange system (model 6400, Li-Cor Instruments, Lincoln, NE, USA) was used to measure the WUE of leaves exposed to full sun in the morning (0900–1100) and in the afternoon (1400–1600). Morning fog or the absence of low hanging branches limited data collection more frequently at Love Valley and Heise resulting in incomplete data sets. Sites with complete WUE data sets (Campo and Cottonwood) were presented.

### 2.5. Statistical analyses

The four morphological characteristics of GSOB infestation and injury were tested for differences among the nine sites. Ratings of the four responses for each tree were summarized for uninfested vs. infested trees and for each site by tree size/age or infestation/injury level. Recently infested trees were analyzed separately from the other sites. Morphological responses to GSOB infestation were also



**Fig. 4.** Ordination of goldspotted oak borer, *Agrilus auroguttatus*, tree health ranking yielded differential loading of the four attributes on each of the three eigenvectors (graphs on the left). Differences in mean ( $\pm 1$  S.E.) eigenvalue for each of the three eigenvectors among uninfested and lightly-, moderately-, and severely-infested coast live oak, *Quercus agrifolia*, levels (on right).

assessed by using principal component analysis (PCA) for all mature and old growth trees across all nine research sites. Principal component analysis was used to determine (1) the relative contribution of each of the four morphological responses to GSOB; and (2) the ability to use the responses to differentiate among tree infestation/injury classes. Statistical analyses of the eigenvalues of mature vs. old growth trees were performed for each of the axes.

Within a site, comparisons between infested and uninfested *Q. agrifolia* of the same size/age class were made with a Student's *t*-test. Statistical significance in morphological and physiological attributes across injury levels and tree sizes (pole, mature, and old growth) was determined by using a one-way analysis of variance. Critical values for significance were reported at the  $\alpha = 0.05$  level. All statistical tests were performed with SigmaPlot 11 (Systat Software, Inc., 2008).

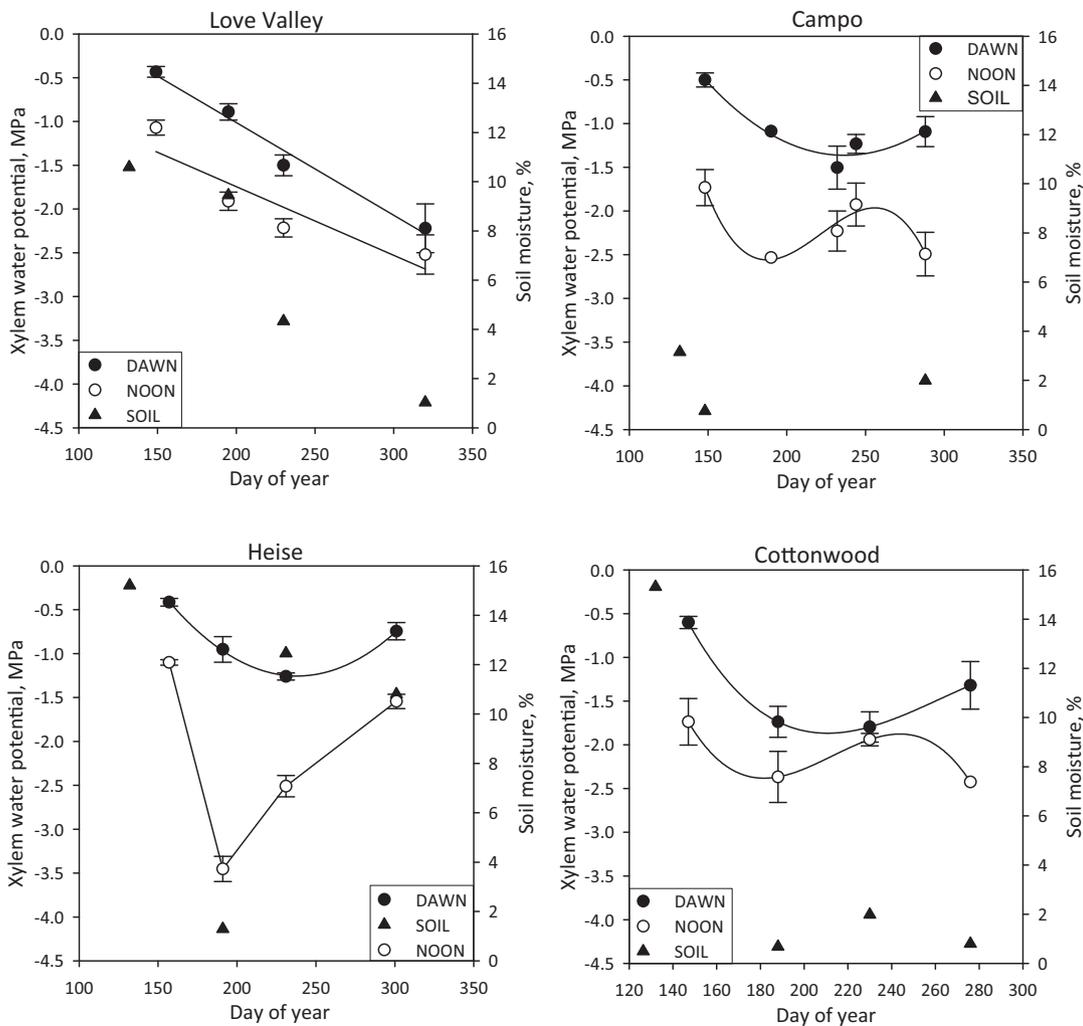
A limited subset of trees with complete data for all attributes was tested for the relationship between morphological and physiological responses by using canonical correlation analysis (CCA). In applying this multivariate analysis, the negative water potentials

were transformed to positive 'dummy variables', then relativized (0–1) to minimize differences among sites. Among the morphological variables, crown thinning and dieback, bole staining, and D-shaped exit holes were tested for correlation with the following physiological variables: early and late summer (growing season) within-day difference in WUE, late summer afternoon WUE, early and late summer  $\Psi_{xPD}$ , late summer  $\Psi_{xSN}$ , and late summer  $\Psi_p$ . The canonical correlation was performed with PC-ORD (McCune and Mefford, 1999).

### 3. Results

#### 3.1. Morphological responses to GSOB injury

Morphological responses to GSOB injury differed among sites, tree size/age classes, and infestation/injury classes (Table 1). In the zone of infestation survey, morphological responses of *Q. agrifolia* did not differ between mature and old growth tree classes. Of these trees, 41% were uninfested and had significantly



**Fig. 5.** Seasonal changes in pre-dawn and solar noon xylem water potential ( $\Psi_{xPD}$  and  $\Psi_{xSN}$ ) for four mature, uninfested trees of coast live oak, *Quercus agrifolia* per site. Soil moisture at 50 cm depth is presented for the same sampling dates from 4 sites for each location.

smaller bole diameter than that of injured trees (Table 1). In both the zone of infestation survey and intensive survey, crown thinness increased with tree size/age class and infestation/injury class for infested trees, and bole stain and exit hole ratings increased with infestation/injury class. The only exception was at the Cottonwood site where crown thinning was lower for moderately-injured trees vs. lightly-injured trees and bark staining was similar for moderately- vs. lightly-injured trees. D-shaped exit holes and woodpecker foraging holes specific to GSOB attack were consistently associated with GSOB injury across all sites (Table 1).

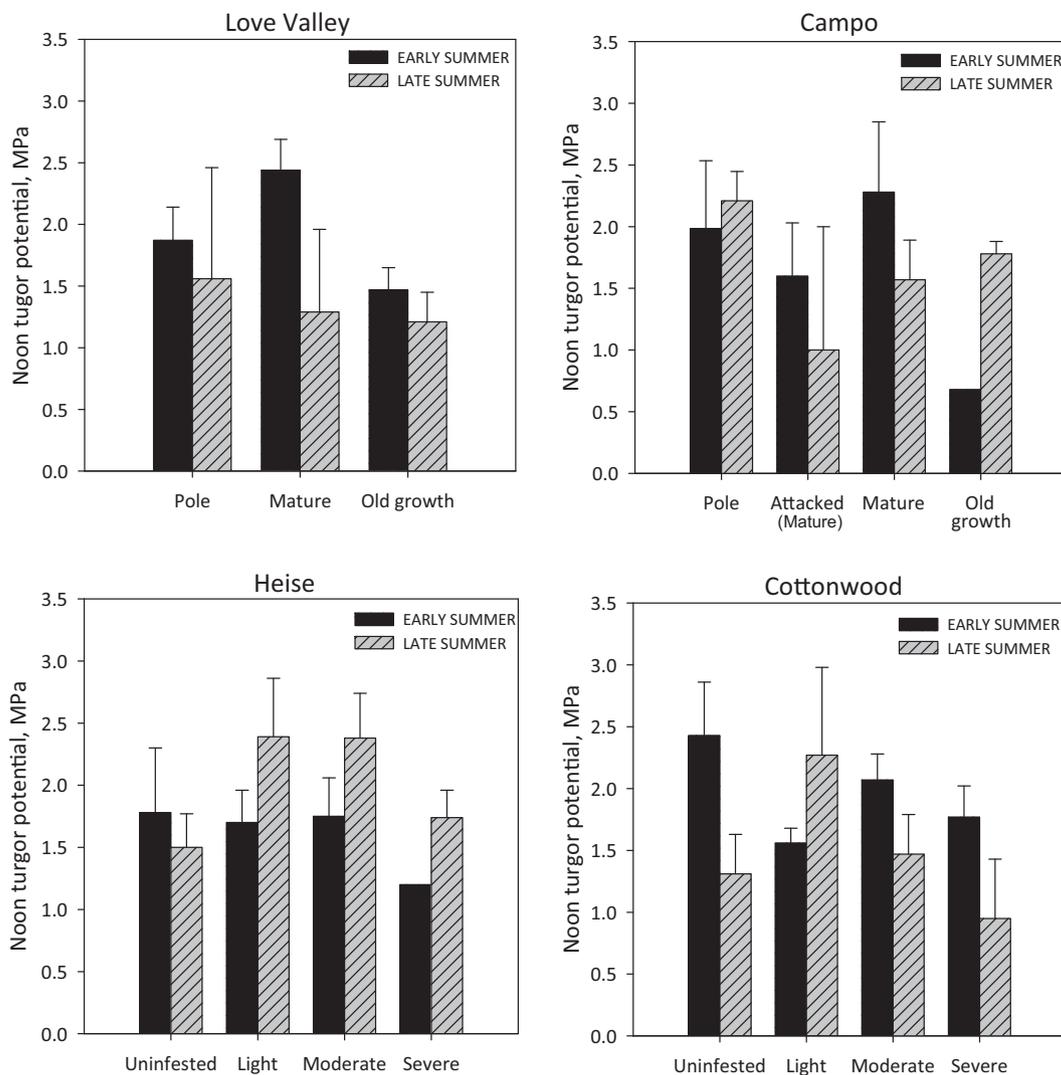
The PCA yielded three significant principal axes, which explained 87% of the variance in the data (eigenvector 1: 48.5%; E2: 23.7%; and E3: 14.8%) (Fig. 4). The first axis loaded equally high on crown thinning, bole staining, and exit hole rating. The second axis loaded highest on the presence/absence of woodpecker foraging holes. The third axis loaded highest on bole staining and exit hole rating. Eigenvalues increased sequentially for injury levels for eigenvector 1. There was high variability in eigenvalues for lightly-injured trees for eigenvector 2 and in all injury levels for eigenvector 3 (Fig. 4).

There were no statistically significant differences between the mature and old growth size/age classes in the first or second axis, but differences were significant in the third axis

( $P < 0.001$ ). Old growth trees exhibited more decline than mature trees.

### 3.2. Physiological responses to drought stress and GSOB injury

Among the mature, uninfested *Q. agrifolia* at the four intensively monitored sites, trees at Heise had the most deleterious physiological response to early summer low soil moisture (<2%), although similar soil moisture levels were measured at Cottonwood on the same date (Fig. 5). Although trees at Cottonwood experienced a similar soil moisture at this time, their solar noon xylem water potential did not decline nearly as much as it did for trees at Heise (Fig. 5). In late summer,  $\Psi_P$  was positive for all mature, uninfested trees at the four sites (Fig. 6). Trees at Campo and Cottonwood had similar seasonal trends in  $\Psi_{xPD}$  and  $\Psi_{xSN}$  (Fig. 5) despite greater early summer soil moisture at Cottonwood. Mature trees at Cottonwood experienced moderate drought stress in mid-July and August, and were unable to recover from low  $\Psi_{xSN}$  by dawn. The lack of recovery would have constituted severe rather than moderate drought stress if positive leaf  $\Psi_P$  had not been maintained (Fig. 6). Mature tree  $\Psi_{xPD}$  and  $\Psi_{xSN}$  at Love Valley declined linearly with soil moisture content from early to late in the growing season (Fig. 5). In late summer, despite significant, regional precipitation events, trees at Love Valley were unable to recover from low  $\Psi_{xSN}$



**Fig. 6.** Noon coast live oak, *Quercus agrifolia*, leaf cell turgor potential ( $\Psi_p$ ) of trees at all sites for early summer (first sampling date corresponding to Fig. 9) and late summer (last sampling date). The height of the bars represents the mean of 3–5 trees in each size size/age or infestation class, with  $\pm 1$  S.E. indicated.

by dawn (Fig. 5). However, late summer leaf  $\Psi_p$  in these trees was still positive (Fig. 6).

Pole-sized, mature, and old growth trees at Love Valley had a similar, linear decline in  $\Psi_{xPD}$  and  $\Psi_{xSN}$  (Fig. 7) with two exceptions. Old growth trees had more favorable  $\Psi_{xPD}$  and  $\Psi_{xSN}$  than did mature and pole-sized trees at the end of the growing season, and there was no recovery by dawn for old growth (or pole-sized) trees. The expression of these attributes constituted moderate, not severe, drought stress (Levitt, 1980) because leaf  $\Psi_p$  was maintained in late summer (across all tree sizes; Fig. 6). Leaf  $\Psi_p$  of pole-sized and mature trees had high within-group variance relative to old growth trees.

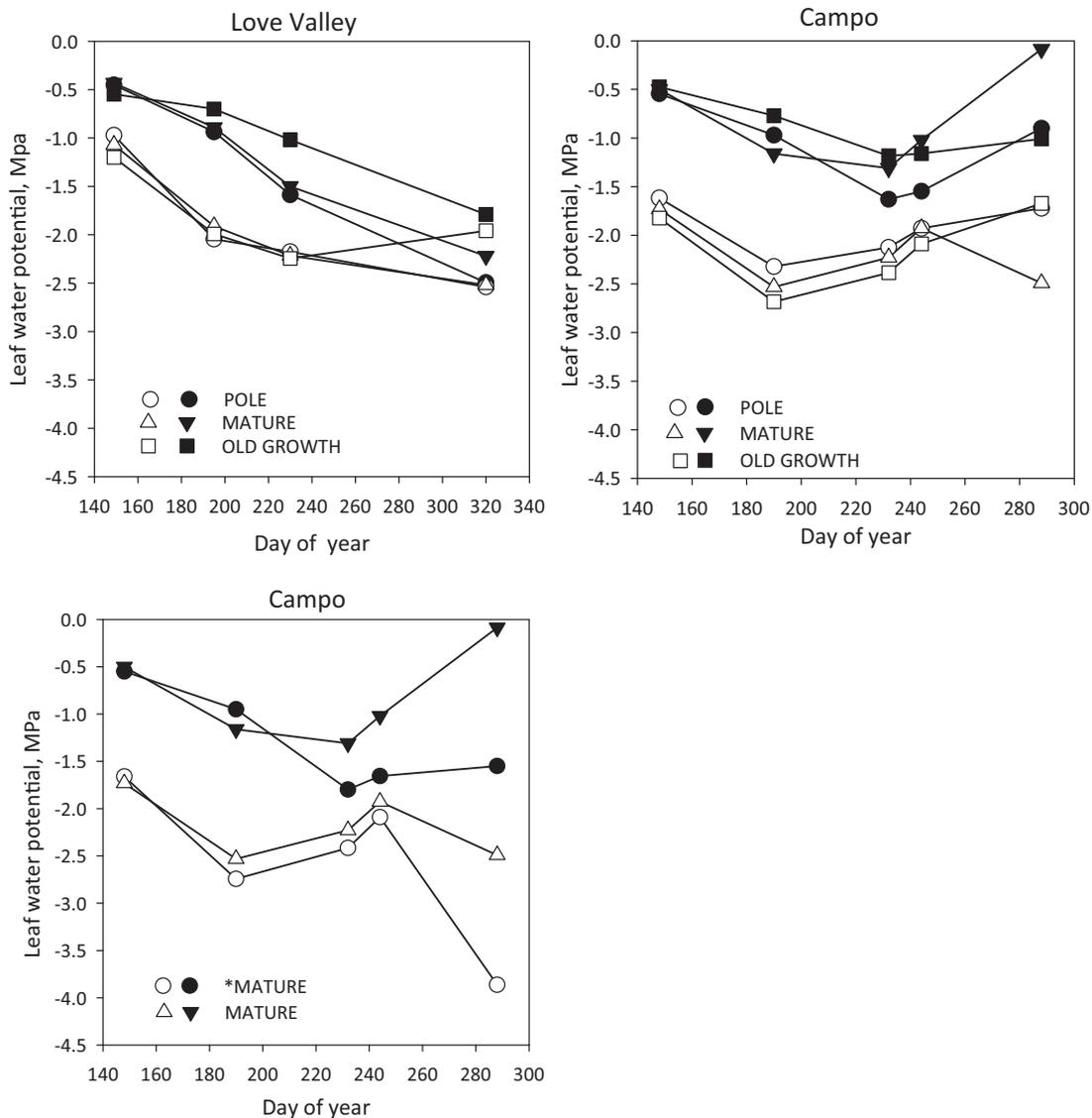
The seasonal patterns of water potential at Campo differed from those at Love Valley. Most trees at Campo showed increasingly favorable  $\Psi_{xPD}$  after the mid-August sampling date (recovery) (Fig. 7). The lowest  $\Psi_{xSN}$  occurred in early July, likely triggering an increase in WUE (Fig. 8). Pole-sized and old growth trees at Campo had similar seasonal patterns in their components of water status (Fig. 7). Among size classes, pole-sized trees had the lowest late summer  $\Psi_{xPD}$  indicating poor overnight recovery from mid-day noon drought stress. However, high  $\Psi_p$  was maintained at a relatively high level throughout the summer (Fig. 6) indicating moderate drought stress (Levitt, 1980). Pole-sized trees at Campo were more drought-stressed in mid-summer than mature and old

growth trees, but had the greatest late summer WUE (Fig. 8). At the end of the growing season, mature trees at Campo had excessively low  $\Psi_{xSN}$ , but recovered fully by dawn (Fig. 7), and  $\Psi_p$  was favorable (Fig. 6). WUE of mature and old growth trees were similar (Fig. 8).

Few differences in leaf  $\Psi_p$  among tree classes were statistically significant due to high within-group variability (Fig. 6). Old growth trees at both Love Valley and Campo had the most favorable plant water status over the whole growing season based on  $\Psi_{xPD}$ ,  $\Psi_{xSN}$ ,  $\Psi_p$ , WUE, and the amount of within-group variance exhibited. Among size classes, pole-sized trees had the highest WUE, but also the highest within-group variance. Of the two larger tree classes, mature trees were the most vulnerable to drought based on high within-group variance of physiological attributes.

There was no progressive difference in  $\Psi_{xPD}$  and  $\Psi_{xSN}$  for trees differing in GSOB injury level at either Heise or Cottonwood (Fig. 9). At Cottonwood in early summer, leaf  $\Psi_p$  declined linearly from uninfested, to light-, moderate-, and severely-injured trees (Fig. 6). In late summer, leaf  $\Psi_p$  was lower for uninfested and severely-injured trees relative to light- and moderately-injured trees.

Only two trees (mature) were infested during the year of study at the Campo site. Qualitative there were no differences in  $\Psi_{xPD}$  and  $\Psi_{xSN}$  of plant water status between infested and uninfested mature trees in early summer, but  $\Psi_{xPD}$  and  $\Psi_{xSN}$  were lower by late summer (Fig. 7).



**Fig. 7.** Comparison of mean seasonal coast live oak, *Quercus agrifolia*, xylem water potential ( $\Psi_x$ ) of small branchlets across tree sizes at uninfested sites (Love Valley and Campo) and for newly infested \*mature trees at Campo. In general,  $\pm 1$  standard error was within the size of the symbol. Closed symbol, pre-dawn, open symbol, solar noon.  $N = 5$  trees per size class.

Pole-sized trees at Campo had greater afternoon than morning WUE in both early and late summer (Fig. 8). Among GSOB injury levels, lightly-injured trees had slightly less favorable WUE than uninfested mature trees at Cottonwood (Fig. 8). Uninfested and lightly-injured mature trees at Cottonwood had greater afternoon than morning WUE in both early and late summer (Fig. 8). In both early and late summer, moderately- and severely-injured *Q. agrifolia* had lower WUE in the afternoon vs. the morning due to low net assimilation in the afternoon (high respiration, data not presented). Mature tree  $\Psi_{xSN}$  at Cottonwood (Fig. 9) did not differ statistically among uninfested, moderately-, and severely-injured trees, nor did noon leaf  $\Psi_P$  differ significantly (Fig. 6). Leaf cell turgor ( $\Psi_P$ ) of old growth trees was the lowest among the three tree size classes at Love Valley and lowest early in the summer at Campo (Fig. 6).

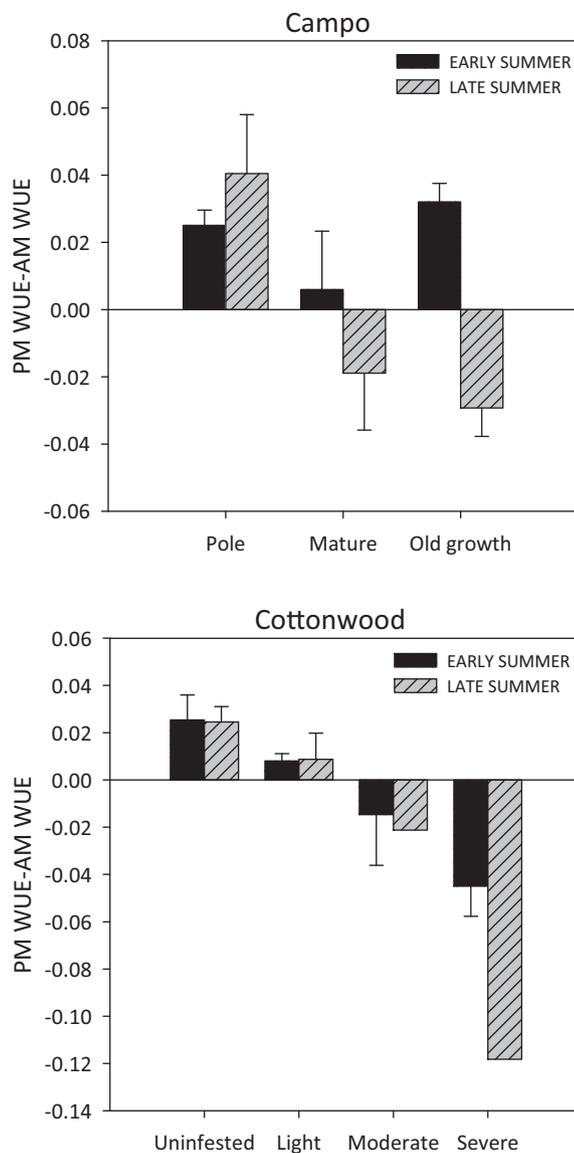
### 3.3. Correlation between morphological and physiological responses to GSOB injury

The CCA had three significant axes, which combined to explain 59.7% of the total variance in the data set (axis 1: 35.0%; axis 2: 15.5%; and axis 3: 9.2%). There was high variability in both mor-

phological and physiological responses, and in the corresponding eigenvalues for the three axes (Fig. 10). Although inclusion of woodpecker foraging holes improved the predictive capability of the PCA, it reduced the predictive capability of the CCA. In the first graph of axis 1 vs. 2, the severely-injured trees were separated statistically from uninfested, and lightly- and moderately-injured trees on axis 1. There was no statistical separation among groups on axis 2. Visually, uninfested and severely-injured trees were spatially separate, and were separate from the combined group (lightly- and moderately-injured trees). In the second graph of axis 1 vs. 3, there was no statistical separation. Uninfested trees were spatially separate from the injured trees.

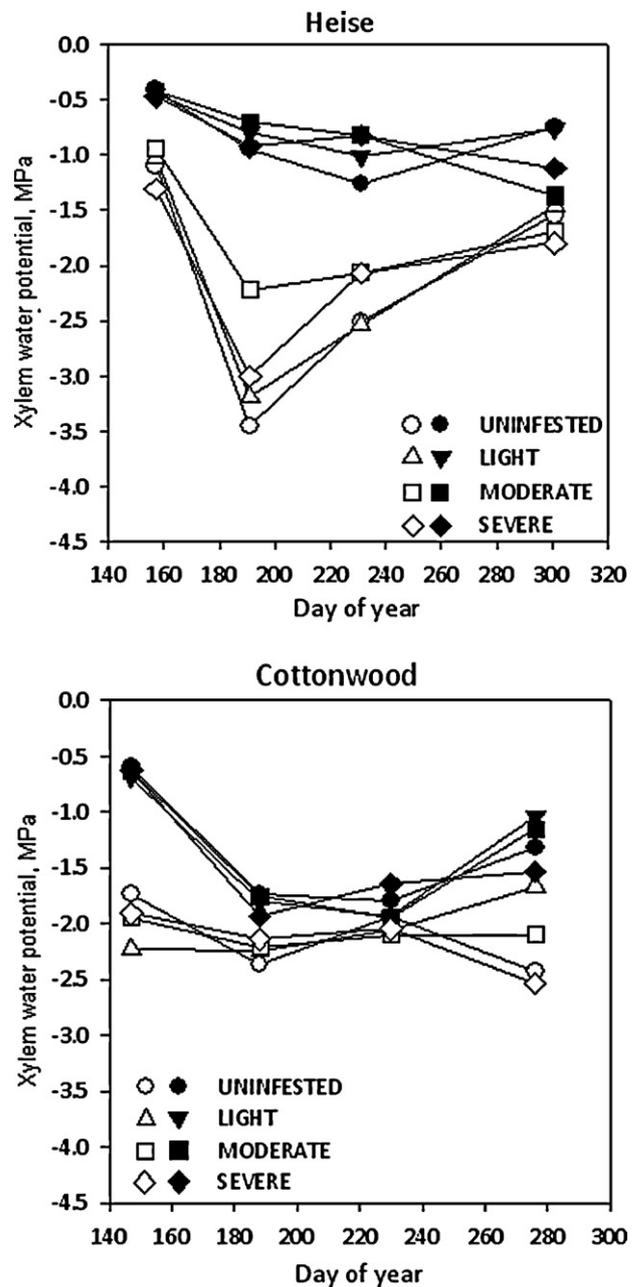
### 4. Discussion

In this study, morphological responses to GSOB injury were defined and evaluated in the context of physiological responses in uninfested and infested *Q. agrifolia*. This analysis was conducted over the course of one growing season to understand the effect of GSOB infestation on tree decline and plant water status. The expression of tree health (e.g., relative frequency and intensity



**Fig. 8.** Seasonal changes in coast live oak, *Quercus agrifolia*, leaf water use efficiency ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). The mean of 3–5 trees is presented, with  $\pm 1$  S.E. The difference between afternoon and morning WUE was plotted for early summer and late summer (growing season) to illustrate differences in strategies for maintaining favorable plant water status.

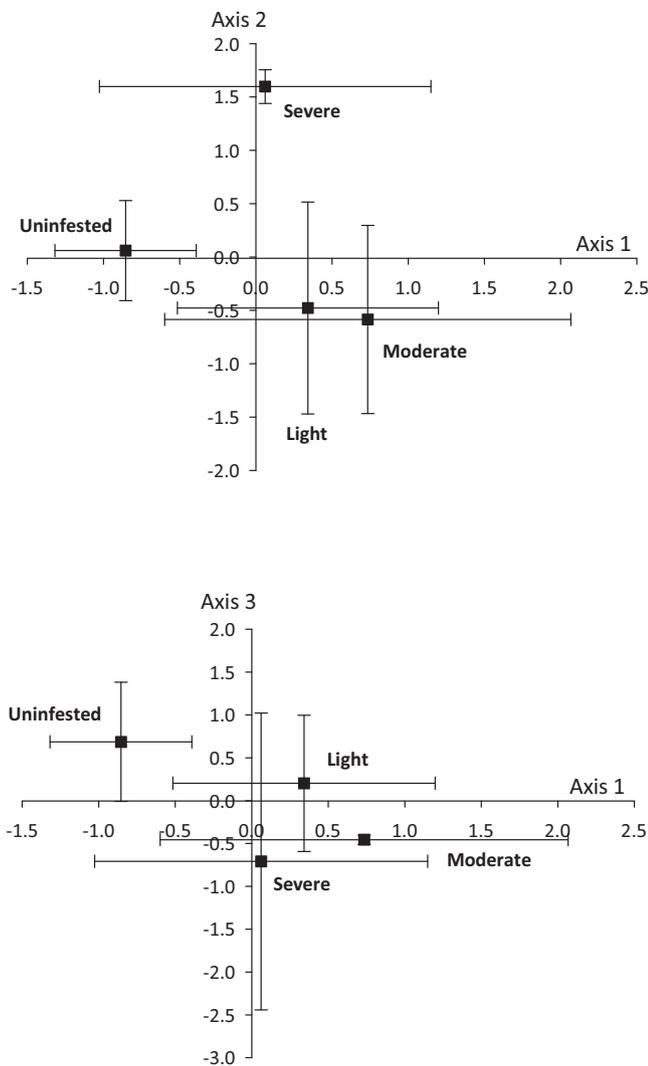
of symptoms within a site) varied across all sites. This could be due to rapid expansion of GSOB and duration that a site has been infested. Environmental and genetic factors may also contribute to site-to-site differences in response of *Q. agrifolia* to GSOB injury, confounding the current classification approach for assessment. For example, crown thinness was the least predictive across GSOB injury levels, and varied significantly among sites (see Table 1, Campo and Love Valley). It is possible that crown condition is too subjective, or a poor predictor, to be used to differentiate among GSOB injury levels (e.g., see axis 3 of Fig. 4). Canopy thinness is a generalized response to many types of stressors. In this study, crown thinness was always associated with moderate to severe GSOB injury, and its use is widespread in both local and national tree health assessments (FIA, Schomaker et al., 2007; EU ICP Forests, Lorenz et al., 2008). A positive relationship was also found between degree of attack from *Agilus biguttatus* and leaf loss (Vansteenkiste et al., 2005). Canopy thinness estimates are linked to biomass and carbon modeling across landscapes. A more quantitative approach



**Fig. 9.** Comparison of coast live oak, *Quercus agrifolia*, xylem water potential (pre-dawn =  $\Psi_{\text{XPD}}$  and solar noon =  $\Psi_{\text{XSN}}$ ) of small branchlets across uninfested and three classes of goldspotted oak borer, *Agilus auroguttatus*, infestation. In general,  $\pm 1$  standard error was within the size of the symbol. Closed symbol, pre-dawn, open symbol, solar noon.  $N = 4$  trees per infestation level.

to estimating canopy thinness will be tested, such as branch demographics (Harper, 1977) or measurement of leaf area index (Law et al., 2001).

Moderately injured trees differed in both stain and exit hole ratings between sites, as did exit hole scoring for severely-injured trees. A more quantitative approach and a method to standardize injury across various size classes for bole staining and exit hole density is being tested. The chronology of these injury responses is not always fixed, but in general, the presence of exit holes is the first sign of injury followed by both foliar loss/crown decline and stem staining. Woodpecker foraging holes can be an early or late symptom of GSOB infestation, and likely the source of high variance in the eigenvalues of lightly-injured trees in the second axis of the PCA.



**Fig. 10.** Canonical correlation analysis of morphological vs. physiological attributes of uninfested and lightly-, moderately-, and severely-injured coast live oak, *Quercus agrifolia*, trees from the goldspotted oak borer, *Agrilus auroguttatus*. N=4 trees per infestation level.

In this assessment, two morphological attributes, exit and woodpecker foraging holes, were definitive for identifying GSOB injury. Due to the rarity of other flatheaded borers injuring oaks along the main stem in California (Brown and Eads, 1965; Swiecki and Bernhardt, 2006; Coleman and Seybold, 2008a, 2011), the size and shape of D-shaped exit holes are diagnostic for GSOB injury. Meandering black colored larval galleries that extend to the outer bark of the main stem, and the presence of pupal cells are also diagnostic for GSOB in California oaks. A low level of bole stain was present on some of the uninfested trees; these trees will be monitored carefully in the future for signs of GSOB injury. Dark-colored, wet staining on the bark, associated with injury of the cambium and accumulated sap, is a common wound response for oak species either from abiotic or biotic factors (Swiecki and Bernhardt, 2006). Sudden oak death, *Phytophthora ramorum*, infection, another serious threat to oak forests in California, is also associated with bark staining or bleeding along the main stem of *Q. agrifolia* (Rizzo and Garbelotto, 2003). Thus, bole staining alone does not define GSOB injury, but the presence of additional injury symptoms or presence of the beetle is needed to validate an infestation.

In this study, few statistically significant differences resulted from the PCA and CCA. Canonical correspondence analysis shows

promise of relating morphological and physiological attributes associated with GSOB injury. The combination of canopy thinness, bole staining, and exit holes was a good predictor for elevated injury GSOB within the PCA.

Based on all of the physiological attributes tested, and considering the level of within-group variance for each attribute, old growth trees maintained the most favorable water status over the growing season, followed by pole-sized trees, then mature trees. Among size classes, pole-sized trees had the highest WUE, but also the highest variance, which suggests vulnerable individuals within the size class (see Grulke, 2010). Along axis 1 of the CCA, the severely-injured trees were spatially separated from uninfested, and lightly- and moderately-injured trees, suggesting fundamental changes in both morphological and physiological attributes of severely-injured trees.

Considering this, and because mature trees also had high variability in most of the physiological attributes, one would expect mature trees to be the most vulnerable to GSOB if initial colonization within a stand occurred on more highly drought-stressed trees. However, old growth trees have been observed to be the first colonized within a stand (Coleman and Seybold, 2008a). In support of this observation, the current study showed that although old growth trees were less common than mature trees, there was a higher proportion of more severely injured old growth trees. The PCA also quantitatively supported greater injury in old growth vs. mature trees in both bole stain and exit holes (Fig. 4). Uninfested mature trees that were similar in size to infested trees at Cottonwood had greater afternoon than morning WUE throughout the summer, representing a typical pattern for vigorous or healthy trees.

Favorable water status detected for old growth trees in the early portion of the growing season and the preference for attack from GSOB on this size class suggest drought stress is not playing a significant role in host selection. Thus, drought stress is not acting as an inciting factor. The aggressive behavior of GSOB in relationship to hosts with relatively satisfactory water relations, stands in contrast to what has been reported for the behavior the twolined chestnut borer, *A. bilineatus*, and the oak splendor beetle, *A. biguttatus* (Haack and Acciavatti, 1992; Moraal and Hilszczański, 2000; Vansteenkiste et al., 2005; Sierpinski and Hilszczański, 2006). We might anticipate even higher levels of tree mortality when predisposing and inciting factors interact with GSOB injury in California in the future (e.g., defoliation of oaks by California oakworm, *Phryganidia californica* Packard).

The explanation for the elevated levels of tree mortality that have been linked to GSOB may have more to do with factors related to its invasion biology in California's oak ecosystem. The apparently increasing trend for populations of forest insects to be re-located or to expand within a continent (i.e., indigenous exotic species) is a worrisome international phenomenon with, in addition to GSOB, recent examples from North America (Dodds et al., 2010); Europe (Pederson and Jørum, 2009) and Asia (Baranchikov et al., 2010). In these instances, seemingly native insects encounter naïve hosts and low population densities of natural biological regulatory factors; hence they behave as exotic species. This presents a political challenge for regulatory agencies within nations.

The two newly injured trees at the otherwise uninfested stand at Campo were both mature trees. Because the trees became symptomatic in early summer when there were no differences in plant water status among size or injury levels at any of the sites, and because there was no data from prior years, we cannot determine whether tree drought stress played a role in their colonization. We will continue to monitor uninfested oaks to determine whether newly infested trees were more drought stressed than other trees within the same size class. The best evidence for the physiological effect of new GSOB injury on *Q. agrifolia* (relative to uninfested

trees) was (1) an 'early warning' of 0.5 MPa lower  $\Psi_{\text{XPD}}$  by mid-August; (2) 1.5 MPa lower  $\Psi_{\text{XPD}}$  and  $\Psi_{\text{XSN}}$  by the end of the growing season (mid-October); and (3) a qualitative reduction in leaf cell turgor ( $\Psi_{\text{P}}$ ) by late summer. This is likely a response, not a predisposition, to GSOB injury.

Oaks have characteristically high variation in physiological attributes (Abrams, 1990). Oaks are drought avoiders or tolerators, and in some species, there is little correlation between gas exchange rates and leaf water potential (Reich and Hinckley, 1989). This was the case in this study: although  $\Psi_{\text{XPD}}$  and  $\Psi_{\text{XSN}}$  were correlated (within a date) and morning and afternoon WUE were correlated, the two types of attributes were not always correlated. When components of total leaf water potential ( $\Psi_{\pi}$ ,  $\Psi_{\text{P}}$ ) are measured ( $\Psi_{\pi}$ ,  $\Psi_{\text{P}}$ ), the level of drought stress can usually be clearly defined. However, there were a number of days when *Q. agrifolia* had net positive carbon gain (despite low conductances), low  $\Psi_{\text{XSN}}$  and no recovery at dawn, yet had positive leaf cell turgor. These species are referred to as regulators (Rambal, 1992) or isohydric (*sensu* Tardieu and Simmoneau, 1998). Within-group variability was not consistent across all physiological attributes at each site. The leaf water potentials that developed over the growing season in this study were considerably lower than those reported for coastal populations of *Q. agrifolia* (at Santa Barbara, CA; Mahall et al., 2009), and intermediate between a summer with  $-2.0$  MPa soil moisture to a summer with  $-4.0$  MPa at 2 m (at Jasper Ridge, CA; Goulden, 1996).

There are four potential explanations for high within-group variability in this study. First, it is possible that GSOB attack initiates a stress response such that lightly attacked trees have a slightly better 'tolerance' for drought stress. This is suggested by the differences in  $\Psi_{\text{XSN}}$  between uninfested and lightly-injured trees, lack of difference between lightly and moderately-injured trees, and  $\Psi_{\text{P}}$  of severely-injured trees in late summer (Fig. 6). For some physiological attributes, there was a polynomial, not linear, response from uninfested to lightly-, moderately-, and severely-injured trees. Initial GSOB colonization may induce a cellular defense that is related to cellular osmoticum, but as the tree declines further, that induction, capacity, or response may be lost. Such an initial response has been observed in herbaceous crops from wounding or fungal attack (Bradley et al., 1992). Second, it is possible that the current year's 'uninfested' trees have just been colonized by neonate larvae and symptoms will develop next year. Third, microenvironmental variability (e.g., soil rooting volume, access to deep water or water in interstices in weathered bedrock; Hubbert et al., 2001; Querejeta et al., 2007) may have as large an effect on physiological status as the level of GSOB injury. We will continue to monitor morphological and physiological attributes of uninfested trees over the next couple of years to try to quantify and assign sources of variability. Fourth, oak branches can abruptly excise entire tertiary or secondary branches within a month as a means to quickly reduce leaf area with developing drought stress. This could be a source of variability in physiological attributes: without a method for determining *a priori* whether the sampled branch would be excised, senescing leaves with lower leaf water potential would be incorporated into late summer samples, increasing within-group variance. In subsequent years of this study, the fate of each secondary branch sampled will be followed for the whole growing season.

There was no progressive difference in  $\Psi_{\text{XPD}}$  and  $\Psi_{\text{XSN}}$  over the growing season for trees differing in level of GSOB injury. However, there were progressive differences among injury levels in WUE over the growing season, and significant changes in the timing of maximum WUE within the day. Because *Q. agrifolia* is an isohydric species, it is likely that despite overall poor crown condition, the remaining leaves maintain reasonably favorable water status and optimize gas exchange for carbon gain until the branch dies.

Pole-sized trees did not appear to be vulnerable physiologically. Pole-sized oaks may be defended from drought stress with higher leaf antioxidants, which help maintain smooth operation of photosynthesis despite environmental stress (Bortier et al., 2000; Tegisher et al., 2002). Lower leaf area: root area relative to mature trees may also help maintain a favorable plant water balance (Mediavilla and Escudero, 2004). Considering all physiological attributes, old growth trees at both Love Valley and Campo had the most favorable plant water status over the whole growing season based on  $\Psi_{\text{XPD}}$ ,  $\Psi_{\text{XSN}}$ ,  $\Psi_{\text{P}}$ , WUE, and the amount of within-group variance exhibited.

Among tree size classes, mature trees had the lowest noon  $\Psi_{\text{P}}$  (Fig. 6), but the highest leaf  $\Psi_{\pi}$  (Fig. 7). In late summer, both mature and old growth trees exhibited a shift in WUE strategy to maintain favorable plant water status: morning WUE was greater than afternoon WUE, primarily due to a higher transpirational load and greater leaf: root ratio (relative to pole-sized trees), and greater respiratory losses in the afternoon. Within the growing season, the lowest  $\Psi_{\text{XSN}}$  occurred in early July, likely triggering an increase in WUE (Fig. 8) (similar to shifts from low to high WUE for ponderosa pine when upper soil horizons dry to 2%; Grulke and Retzlaff, 2001). Old growth trees were the first to be colonized by GSOB and exhibited the lowest leaf  $\Psi_{\pi}$  (but not the lowest noon  $\Psi_{\text{P}}$ ) among the tree size classes. Old growth trees had the greatest change in WUE from early to late summer. Old growth oak trees have a greater requirement for both water (due to leaf area and high leaf: root surface area) and photosynthates (due to maintenance requirements for their extensive structural support) (Magnani et al., 2000; Niinemets, 2002). With the experimental plots outlined here, we plan to improve the quantitative basis of the tree health rating system, but also continue to investigate the relationship between plant water status and initial host colonization by GSOB, symptom development and physiological response, rate of spread within a stand, and the rate of tree decline to mortality.

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